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RADIATION-INDUCED INHIBITION OF ECLOSION IN THE TOBACCO HORNWORM, *MANDUCA SEXTA*

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Increases in radioresistance in *Drosophila melanogaster* during pupal-adult development were first observed by Mavor (1927) and have since been documented for a number of holometabolous insects (Grosch, 1962 and 1973; Arena, 1971). Mavor (1927, p. 81) concluded that, ". . . the resistance of the pupae to X-rays begins after all of their important organs have been laid down and that their resistance increases with the growth and differentiation of these organs." Preliminary investigations with *Manduca sexta,* X-irradiated at various intervals during pupal-adult development, indicated an increase in radioresistance during this developmental period (Ely and Jungreis, 1976, 1977). Of particular interest in these studies was the observation that X-irradiated animals which failed to eclose were still able to complete metamorphosis.

In the normative scheme of insect development, the sequence of molts and morphological changes characteristic of post-embryonic development is controlled by the interaction of hormones produced by components of the neuroendocrine system (see Wyatt, 1972; Doane, 1973). In holometabolous insects, the last major developmental event in the life cycle is eclosion. Hormonal control of eclosion has been demonstrated in at least three species of silkworms, namely *Hyalophora cecropia, Antheraea polyphemus* and *Antheraea pernyi* (Truman and Riddiford, 1970). Utilizing "loose-brain" animals and brain homogenates, these investigators concluded that the brain was the site of eclosion hormone production. Furthermore, the release of hormone is synchronized with photoperiod regimen and acts on the central nervous system to elicit a species-specific pattern of behavior (pre-eclosion behavior). It is this elicited behavioral response which results in rupture and ecdysis of the pupal cuticle. Pre-eclosion behavior patterns are programmed in the abdominal ganglia rather than the brain (Truman, 1971; Truman and Sokolove, 1972). Eclosion hormone thus serves to trigger the sequence of abdominal movements necessary for emergence. Utilizing pharate adult *A. pernyi* in an assay for eclosion hormone activity, Truman (1973) found high concentrations of the hormone in the brains and corpora cardiaca of *M. sexta* pharate adults on the day of eclosion and provided evidence that the cells in the median neurosecretory cluster of the brain were responsible for eclosion hormone production. Therefore, it is postulated that the radiation-induced inhibition of eclosion observed in *Manduca* results from a disruption of the normal hormonal mechanisms controlling adult emergence.

Studies herein described were undertaken to both analyze in greater detail the effects on adult emergence of X-irradiation of pharate-adults of *M. sexta,* and to assess the neuroendocrine contribution, if any, to observed changes in radiosensitivity exhibited by this species during the pupal-adult transformation.

Materials and Methods

Experimental animals

Tobacco hornworms used in these experiments were derived from an inbred colony maintained by Dr. Lynn M. Riddiford, University of Washington, Seattle. Larvae were individually reared in plastic containers on the diet of Yamamoto (1969), modified after Bell and Joachim (1976) and Riddiford (personal communication), under an 18L : 6D photoperiod regimen at 23–25° C, according to the methods outlined in Ely and Jungreis (1977).

Irradiation parameters

The X-irradiation source was a General Electric Maxitron 300 X-ray unit operated at 250 kV and 15 mAmp with 0.5 mm aluminum filtration added (4.75 mm Be inherent filtration) to reduce tissue attenuation by 95–100%. Exposures were altered by varying the time of exposure and/or the target-to-object distance. Dosimetry, as measured in air, was determined under experimental conditions utilizing either a 250-r thimble ionization chamber read in a Model 70 Victoreen condenser r-meter, or a Model 575 Victoreen Radocon with medium energy (Model 602) probe.

Animals receiving whole-body or partial-body exposures (in the case of shielding studies) were positioned on a styrofoam platform, 2.5 cm in thickness, during irradiation. Following treatment, all animals were returned to laboratory rearing conditions.

Determination of ED_{50}'s

Animals were divided into three to five treatment groups (25 animals per group) and a control group (25 animals) on each day of the pupal-adult transformation. Treatment groups were given a series of graded whole-body exposures at an exposure rate of approximately 325 r/minute. The range of exposures to which animals were subjected on any given day during development was based on preliminary investigations of radiosensitivity. The fraction of irradiated animals eclosing relative to nonirradiated controls was plotted as a function of exposure and the ED_{50} (X-ray exposure required to prevent 50% of irradiated animals from emerging) determined for each day of pupal-adult development utilizing regression analysis. In this work, eclosion will be defined as those events associated with the emergence of an imago from the confines of the pupal exuvia following completion of pupal-adult development.

Body shielding experiments

Animals were subjected to partial-body irradiation on the tenth day after pupation (day 10) at an ED_{100} level of 48 kr (325 r/minute). A lead plate, 1 cm in thickness, was placed over selected body regions. Exposures in the shielded regions were approximately 1–2% of those measured in the unshielded regions.

Brain transplantation experiments

In a selected group of pharate adults anesthetized with water saturated carbon dioxide (for up to 30 minutes), the brains (supra-esophageal ganglia) were surgically manipulated following procedures slightly modified after Williams (1959). (See Eaton (1974) and Eaton and Dickens (1974) for descriptions of the topography of the brain and central nervous system of *M. sexta*.) A section of cuticle (approximately 2.5 mm by 2.5 mm) was removed from the dorsal regions of the head, and the brain (or brain-corpora cardiaca complexes) either removed or left in place after severing the major nerve trunks leading to the brain. Surgical procedures were carried out with the aid of a dissecting microscope on both non-irradiated animals, and on animals which had just been irradiated with an ED_{100} exposure of 48 kr delivered at 325 r/minute (see Ely and Jungreis, 1977). Prior to each group of operations, dissecting instruments were rinsed in 95% ethanol. Following surgery, a few crystals of an equal part mixture of phenylthiourea and streptomycin sulphate were placed in the wound. Sterile physiologic saline (35 mM KCl, 5 mM $MgCl_2$, 5 mM $CaCl_2$, 200 mM sucrose) was then added to the haemocoel until the level of hemolymph was flush with the surface of the cuticle. The opening was then covered with a plastic window (cut from cellulose acetate cover slips rinsed in 95% ethanol) and sealed in place with hot paraffin wax. Animals were then returned to the insectory. After 24 hours, pharate adults were checked for the presence of trapped air. If air bubbles were visible through the plastic window, the wound was opened and resealed as described above.

Intact brains were implanted into the abdomens of treated and control animals by cutting a small opening in the next to last abdominal segment and inserting the tissue. Procedures for abdominal cuticle excision and wound closure were similar to those described above for head surgery.

Ecdysone administration

Anhydrous 20(R)-hydroxyecdysone (β-ecdysone) (Sigma Chemical Corporation) was diluted with sterile 0.1 M KCl saturated with phenylthiourea to give a solution having a final concentration of 1 microgram ecdysone per microliter.

Animals were individually weighed, anesthetized with carbon dioxide, and injected by means of a 25 microliter Hamilton syringe with β-ecdysone through the mesothoracic tergum on days 1, 4, and 12 of pharate-adult development. The injection site was then sealed with paraffin wax and the animals returned to laboratory rearing conditions. The quantity of β-ecdysone injected was based upon the volume of hemolymph which was assumed to be 40% of body wet weight. This value was used rather than the 50% determined for *H. cecropia* (Jungreis and Wyatt, 1972) or *Antheraea pernyi* (Cherbas and Cherbas, 1970) because of the greater contribution of the pupal cuticle in *M. sexta* to the body weight.

Following administration of ecdysone, animals were irradiated as follows. First, day 1 animals receiving 4 μg of β-ecdysone per gram of wet weight were X-irradiated at ED_0 (see Ely and Jungreis, 1977) with an exposure of 8.23 kr (325 r/minute) at 6, 24, and 48 hours post-injection. One group of animals, injected on day 1 with 4 μg ecdysone per gram of wet weight, received a second ecdysone injection (again, 4 μg/gram wet weight) 24 hours after the first. This

group was then subjected to an 8.23 kr X-ray exposure (325 r/minute) 24 hours following the second ecdysone administration. Day 1 animals receiving 4 μg ecdysone/gram wet weight without subsequent irradiation served as a control group. Secondly, day 12 animals were treated in a manner identical to that used for day 1 animals with the exception that the ED_0 X-irradiation exposure was 25 kr (325 r/minute). Thirdly, day 4 animals were treated in a manner similar to day 1 animals except that at 6, 12, 24, 36, and 48 hours post ecdysone injections the animals were irradiated at an ED_0 level. Fourthly, day 4 animals were X-irradiated with an exposure of 8.23 kr (325 r/minute) 24 hours following administration of 2, 4, 6, 12, and 16 micrograms of ecdysone per gram of wet weight.

Eclosion success was determined for each experimental group and expressed as a percentage of eclosion success observed in noninjected animals irradiated at similar time intervals. Day 4 and day 12 data were analyzed using single classification analysis of variance. The Student-Newman-Keuls test was employed for a comparison among means (Sokal and Rohlf, 1969).

Results

Effects of X-irradiation on eclosion

The effect of X-irradiation on eclosion of *M. sexta* during the pupal-adult transformation was investigated by initially determining the ED_{50} for animals on each day of development and by utilizing the ED_{50} values as a relative measure of radiation sensitivity (Fig. 1). An initial period of nearly constant radiosensitivity

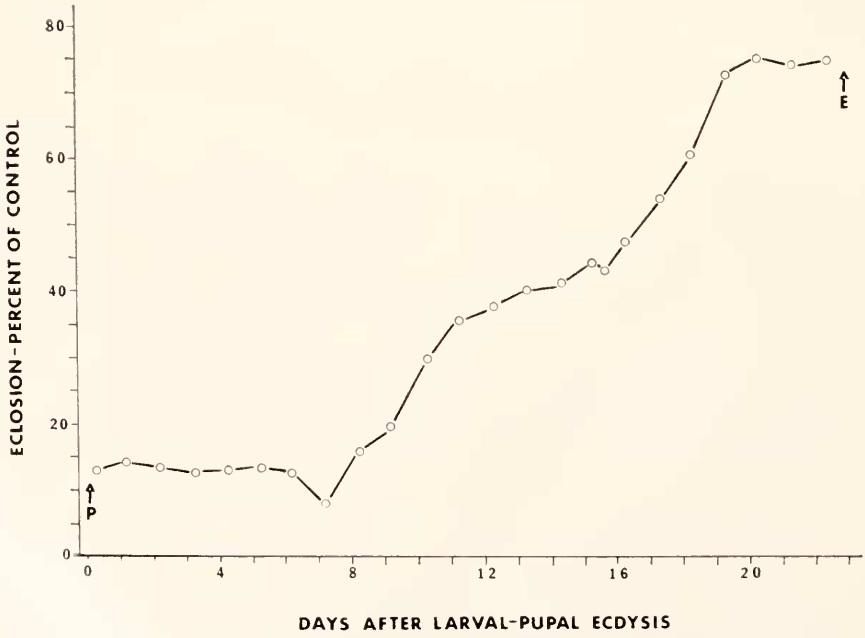

Figure 1. ED_{50} values (X-ray exposures required to prevent eclosion in 50% of the irradiated animals) during pupal-adult development of *Manduca sexta*.

was noted between days 0 and 6 (ED_{50}'s ranged from 13.0 kr on days 0 and 6 to 14.4 kr on day 1). This period of constant sensitivity was followed by a brief period of increased radiosensitivity (ED_{50} on day 7 of 8.23 kr) and in turn followed by a pronounced decrease in radiosensitivity through the day of eclosion (day 8: $ED_{50} = 19$ kr; day 22: $ED_{50} = 75$ kr).

The timing of maximum sensitivity on day 7 was determined with greater precision by X-irradiating pharate adults at an exposure level of 8.23 kr (the ED_{50} for day 7) at 6-hour intervals between day 6 and day 8. The increasing radiosensitivity begins 6.25–6.50 days following pupation, reaches a maximum level between 7.25 and 7.50 days, and then returns to a level approximating that seen 6.0–6.25 days following pupation (Fig. 2).

Brain extirpation/transplantation experiments

The role of the brain as a radiosensitive component in animals following the period of maximum radiosensitivity (*i.e.*, day 7) was investigated following brain extirpation and brain transplantation experiments performed on irradiated (with an ED_{100} exposure of 48 kr) and nonirradiated day 10 animals.

Debraining of nonirradiated animals resulted in only 3% eclosion success (Table I). Clearly, the brain is required for successful adult emergence. However, connectives leading from the brain to other regions of the central nervous system must also be intact if eclosion is to occur, since cutting the connectives to the brain of nonirradiated animals resulted in only 45% eclosion success.

Irradiation of day 10 animals with an exposure of 48 kr (ED_{100} exposure) resulted in 0% eclosion success. Implantation of nonirradiated brains into the abdomens of ED_{100} animals did not improve eclosion success, while removal of

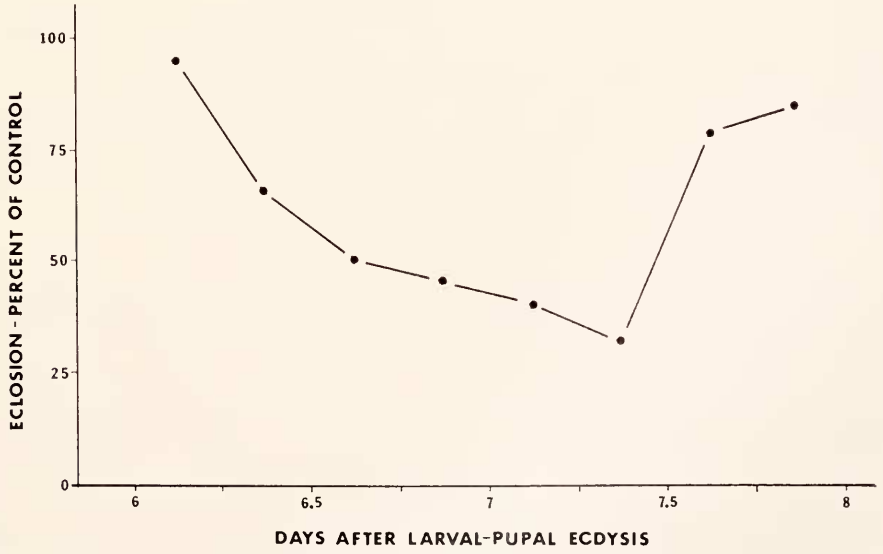

FIGURE 2. Eclosion of *Manduca sexta* X-irradiated during pupal-adult transformation with an exposure of 8.23 kr; 25 animals per exposure group.

TABLE I

Eclosion of Manduca sexta *following surgical manipulation of the brain and/or X-irradiation with an* ED_{100} *exposure of 48 kr on day 10 of pupal-adult development.*

Group number	Treatment	Eclosion	
		Number	Per cent
	Nonirradiated animals		
1	No surgical manipulation	20/20	100
2	Debrained	1/31	3
3	Debrained; nonirradiated brain implanted into head	9/20	45
4	Debrained; ED_{100} brain implanted into head	1/15	7
5	ED_{100} brain implanted into abdomen of animal whose own brain intact	15/15	100
6	Nerve connections to the brain severed	9/20	45
	ED_{100}-irradiated animals		
7	No surgical manipulation	0/20	0
8	Debrained following irradiation; nonirradiated brain implanted into head	1/15	7
9	Nonirradiated brain implanted into abdomen of animal whose own brain was intact	0/20	0

brains from ED_{100} animals following exposure with subsequent implantation of nonirradiated brains resulted in only 7% eclosion success.

This suggests that radiation damage to the brain alone cannot completely account for the 0% eclosion success observed in Group 7 of Table I. However, it is also clear that the brain is "damaged" by irradiation, and that this damage reduces the chance for successful eclosion, as evidenced by comparing the results obtained from Group 3 and Group 4 in Table I. Adding nonirradiated brains to debrained, nonirradiated animals results in a 45% eclosion success. Adding brains irradiated at the ED_{100} level to debrained, nonirradiated animals, however, results in only 7% eclosion success. The 100% eclosion success observed in Group 5, Table I, suggests that the decrease in emergence noted in irradiated animals cannot be attributed to an "active inhibition" of eclosion by the irradiated brain itself.

To determine whether radiation-induced brain damage was accompanied by a decrease in eclosion hormone activity in brains and corpora cardiaca of X-irradiated *M. sexta* pharate adults, animals irradiated with an ED_{100} exposure of 48 kr on day 10, along with nonirradiated animals, were sent to Dr. James W. Truman, University of Washington, Seattle, for biological assay of eclosion hormone (Truman, 1973). In the X-irradiated group of animals, 46% of the brains and 31% of the corpora cardiaca assayed positive for eclosion hormone. This is in marked contrast to the nonirradiated group in which 75% of the brains and 64% of the corpora cardiaca gave positive responses indicating the presence of eclosion hormone activity.

Effects of body shielding on eclosion success

Partial body irradiations of day 10 animals, utilizing an ED_{100} exposure of 48 kr, were performed to identify sensitive and tolerant body regions. Eclosion success following selective shielding is summarized in Table II.

Whole body exposure at 48 kr resulted in 0% eclosion success. Shielding of the abdomen alone resulted in 10% emergence, while 100% of the animals whose head and thorax were simultaneously shielded during irradiation successfully eclosed.

Comparison of Groups 2 and 3 with Group 5 of Table II indicates that the improved eclosion success observed in these former groups can be attributed to shielding of the thorax (Group 2) or shielding of the head (Group 3). Since shielding of the head alone (Group 4) results in 30% eclosion success, it appears that, of the three body regions shielded, the thorax is the most radiosensitive, with the abdomen being least sensitive (Group 1), and the head region being of intermediate radiosensitivity.

Effects of β-ecdysone on eclosion

The possible association between the maximum period of radiosensitivity observed in day 7 animals and an elevated level of endogenous ecdysone (see Kaplanis, Thompson, Yamamoto, Robbins, and Louloudes, 1966) was investigated by injecting day 1, day 4, and day 12 animals with 4 micrograms of β-ecdysone per gram of wet weight and irradiating at specific intervals post-injection with the maximal level of radiation that failed to interfere with eclosion (ED_0 exposure). β-Ecdysone has previously been shown to be the active form of the molting hormone controlling post-embryonic growth and development in tobacco hornworms (Kaplanis *et al.*, 1966; King, Bollenbacher, Borst, Vedeckis, O'Connor, Ittycheria, and Gilbert, 1974).

Administration of ecdysone to days 1, 4, and 12 animals, without subsequent irradiation, resulted in mean eclosion successes of 87%, 87%, and 93%, respectively (Table III). Significant increases in radiosensitivity ($P < 0.05$) were noted in day 4 animals 12 and 24 hours post-injection; and in day 12 animals 6 hours post-injection. Irradiation of day 1 animals following a double-injection routine also increases radiosensitivity as indicated by 53% eclosion success.

TABLE II

Eclosion following partial-body X-irradiation of day 10 Manduca sexta *with an exposure of 48 kr.*

Group number	Body region shielded	Eclosion	
		Number	Percentage of control
1	Head and thorax	20/20	100
2	Thorax and abdomen	18/20	90
3	Head and abdomen	14/20	70
4	Head only	6/20	30
5	Abdomen only	2/20	10
6	No shielding	0/20	0

TABLE III

Eclosion of day 1, day 4, and day 12 Manduca sexta *pharate adults X-irradiated with ED_0 exposures of 8.23 kr, 8.23 kr, and 25 kr, respectively, following injection of 4 micrograms of β-ecdysone per gram wet weight. Results are expressed as percentages of noninjected, irradiated controls.*

Treatments	Eclosion				
	Day 1 animals	Day 4 animals		Day 12 animals	
		Series 1	Series 2	Series 1	Series 2
Injected, nonirradiated	87 (13/15)	80 (12/15)	93 (14/15)	93 (14/15)	93 (14/15)
Irradiated at the following intervals post-injection:					
6 hours	87 (13/15)	60 (12/20)	87 (13/15)	40 (6/15)	53 (8/15)
12 hours	—	30 (6/20)	40 (6/15)	–	—
24 hours	73 (11/15)	47 (7/15)	55 (11/20)	87 (13/15)	100 (15/15)
36 hours	—	72 (13/18)	73 (11/15)	67 (10/15)	40 (6/15)
48 hours	93 (14/15)	100 (15/15)	93 (14/15)	60 (9/15)	47 (7/15)
Irradiated 24 hours following double injection	53 (8/15)	67 (10/15)	60 (9/15)	60 (9/15)	73 (11/15)

The degree of radiosensitivity observed in day 4 animls irradiated 24 hours post-injection is clearly dependent upon the quantity of ecdysone administered (Fig. 3). All animals irradiated 24 hours following injection of 12 and 16 micrograms of

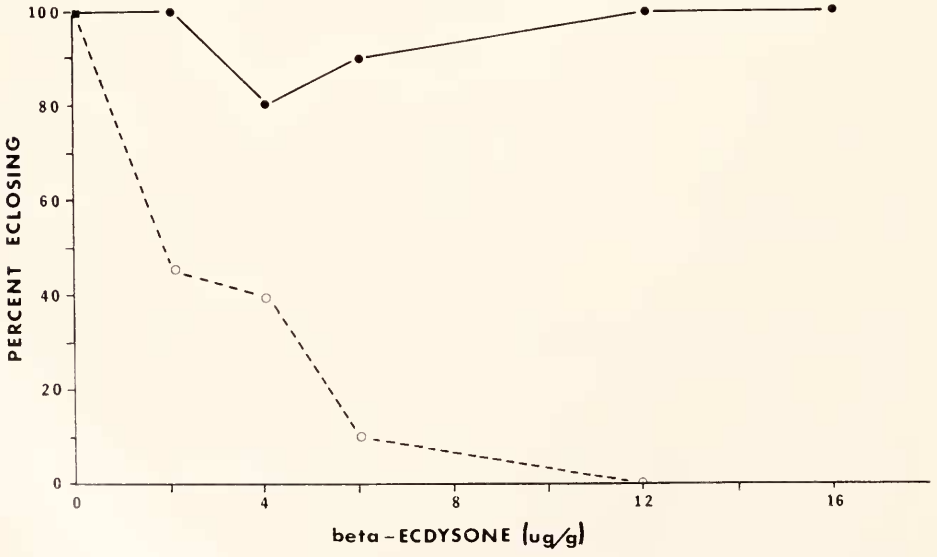

FIGURE 3. Eclosion of *Manduca sexta* following injections of β-ecdysone on day 4 of pupal-adult transformation: (A) nonirradiated; (B) X-irradiated with an exposure of 8.23 kr 24 hours post-injection; 20 animals per treatment group.

ecdysone per gram of wet weight failed to eclose while those receiving identical ecdysone doses but not irradiated exhibited 100% eclosion success.

Discussion

Evaluating the action of X-irradiation of *Musca domestica* pupae upon eclosion, Rockstein, Dauer and Bhatnager (1965) proposed two possible mechanisms to account for the reduced emergence of irradiated flies; namely, premature termination of pupal-adult development, and interference with the actual process of eclosion possibly brought about by damage to the muscle systems involved in the rupture and shedding of the pupal cuticle. These investigators considered the first mechanism to be of minor importance over the exposure range utilized for two reasons; namely, that unemerged, irradiated puparia contained fully formed adults, and that partial eclosion was observed in some animals. Preliminary investigations with *M. sexta* indicated that X-irradiation during the pupal-adult transformation normally inhibited eclosion without disrupting the pupal-adult metamorphosis (Ely and Jungreis, 1977).

In *H. cecropia, A. pernyi,* and *A. polyphemus,* specific hormones are involved in eclosion (Truman and Riddiford, 1970). Therefore, it was initially assumed that the pattern of radiosensitivity (as measured by the ED_{50} values) in *M. sexta* X-irradiated during the pupal-adult transformation would reflect changes in the radiation sensitivity of some component or phase in hormone production. Were the control of eclosion hormone synthesis and release in *M. sexta* similar to that of *A. pernyi* (Truman, 1973), then the observed pattern of radiosensitivity might reflect damage of neurosecretory cells responsible for eclosion hormone production, storage and/or release. To date, a quantitative assay for measuring the titer of eclosion hormone in *M. sexta* has not been reported. Therefore, a direct correlation between radiosensitivity and eclosion hormone levels can not be made.

The titers of ecdysone during pharate-pupal and pharate-adult development have been measured in the tobacco hornworm by several investigators (Kaplanis, *et al.*, 1966; Bollenbacher, Vedeckis, Gilbert and O'Connor, 1975). Employing a biological assay system, Kaplanis and co-workers (1966) monitored changes in ecdysone levels during pupal-adult development in hornworms reared at $24 \pm 2°$ C (eclosion on day 21) and noted a single peak with the maximum titer occurring 6–8 days after the larval-pupal ecdysis. Since the period of maximum ecdysone titer coincided with that of maximum radiosensitivity (day 7) (determined *via* ED_{50}'s), the existence of a positive relationship between these variables could be inferred. The observed relationship between ecdysone and eclosion hormones can be explained in at least two ways; namely, that synthesis of β ecdysone is interrupted by radiation exposure, and that the increased presence of ecdysone renders the animal more sensitive to the effects of radiation. The first model is clearly incorrect, since 93% of irradiated day 7 animals which did not eclose were noted to have completed pupal-adult development. The time course of this development was identical to that observed in nonirradiated animals. Observed increases in radiosensitivity in day 1, day 4, and day 12 animals injected with β-ecdysone (Table III) provide support for the second model. However, the temporal pattern of response in these groups may be dependent upon both the competence of the animal to respond to exogenous β-ecdysone and the titer of endogenous

β-ecdysone at the time of injection and subsequent irradiation. For example, whereas increases in radiosensitivity in days 4 and 12 animals occurred 12 hours and 6 hours post-injection, respectively, that in day 1 animals was elicited only after a double injection of ecdysone (Table III). In this regard, Kaplanis and co-workers (1966) found that the titer of ecdysone in day 1 animals is only 60% of that found in day 4 and day 12 animals. Since the observed radiosensitivity induced by exogenous ecdysone appears to be dose-dependent, then the *total* quantity (both exogenous and endogenous) of β-ecdysone present in hemolymph would be substantially lower in the day 1 groups receiving single injections relative to day 4 and day 12 groups.

The mode of action of ecdysone in effecting the increase in radiosensitivity was not investigated directly. One explanation involves the possible role of ecdysone as a messenger responsible for "turning on" eclosion hormone production in the brain. Irradiation of the brain following ecdysone stimulation could interfere with those critical biochemical activities (such as mRNA synthesis) required for eclosion hormone synthesis, storage, and release.

Eclosion hormone in *M. sexta* is thought to be synthesized by the median neurosecretory cells of the brain and stored in the corpora cardiaca prior to its release into hemolymph at the time of eclosion (Truman, 1973). After initially demonstrating that a hormone stored in the brain was responsible for eclosion in the silkmoth, *H. cecropia,* Truman and Riddiford (Truman and Riddiford, 1970, 1974; Truman, 1971) observed that its presence was not an absolute requirement for eclosion, since animals debrained during diapause or on days 0–3 of pharate adult development were still able to eclose. However, the coordinated preeclosion behavioral pattern characteristic of this species was observed in only 12% of such surgically manipulated animals.

Working with hibernally diapausing *M. sexta,* Judy (1972) reported that animals debrained 20 to 50 days after the larval-pupal ecdysis were able to terminate diapause and complete adult development, but not successfully eclose, observations consistent with those recorded in Table I. In apparent contrast to these results, Wilson and Larsen (1974) observed a 70% rate of eclosion success in *M. sexta* pupae, whose brains were removed at selected early, middle, and late pupal stages. Since Judy (1972) and Wilson and Larsen (1974) debrained *M. sexta* at different stages in development, their results are not comparable.

The role of the brain in regulating eclosion in *M. sexta* was determined by carrying out brain extirpation and exchange experiments in conjunction with selective shielding studies on irradiated and nonirradiated animals at day 10 of pupal-adult development. The eclosion success observed in debrained, nonirradiated day 10 animals was only 3%, a value in marked contrast to the 70% observed by Wilson and Larsen (1974). This finding is also at variance with results obtained with debrained *H. cecropia* (Truman, 1971), but is consistent with results obtained by Judy (1972) for *M. sexta.* Furthermore, the 45% eclosion success seen in animals following simple brain exchange and following severence of the major nerve trunks to the brain indicates that the role of the brain in controlling eclosion cannot be explained entirely in terms of hormonal mechanisms. For example, the zero level eclosion success observed in day 10 animals following a 48 kr X-ray exposure is not merely the result of radiation damage to

the brain, since implantation of nonirradiated brains into the heads of animals debrained following irradiation or implantation of nonirradiated brains into the abdomens of animals whose own brains remained intact did not significantly improve eclosion success. Had the brain been the sole target of radiation damage, one would have expected an eclosion success of at least 45% in each of these groups. The 7% eclosion success observed in nonirradiated, debrained animals into which ED_{100} brains were implanted clearly demonstrates that radiation damage affecting eclosion does occur to the brain. Were this not the case, a 45% eclosion success would also have been expected in this group.

In an attempt to identify radiosensitive body regions without resorting to surgical manipulation, selected irradiations at the ED_{100} level of day 10 animal body regions were performed (see Table II). Little enhancement in eclosion success was observed in abdomen-shielded animals. This suggests that any radiation damage in this body region, including damage to the abdominal neurocircuitry and musculature involved in eclosion behavior, does not significantly decrease the chances for successful emergence. Simultaneous shielding of the head and thorax, on the other hand, resulted in a 100% eclosion success. Furthermore, these data suggest that the relative contribution of shielding the thorax is greater than the head shielding in decreasing radiation-induced inhibition of eclosion. Again, radiation damage to the head (including the brain), although a contributory factor, cannot fully account for the decrease in eclosion success observed in these experimental groups.

Truman (1973) has shown that the information required for the nonrepetitive sequence of motor acts involved in eclosion is built into components of the central nervous system which reside outside of the brain. The possibility thus exists that the extensive radiation damage to these components could result in an inhibition of eclosion even should a sufficient quantity of eclosion hormone be present. Williams (1969) has examined the contribution of the thoracic and abdominal ganglia to pupal-adult morphogenesis and emergence using denervated silkworm pupae (*H. cecropia* and *A. polyphemus*). Pupal excision of the central nervous system (brain, subesophageal ganglion, thoracic ganglia, and abdominal ganglia) resulted in termination of adult development. Implantation of loose brains from chilled pupae into such denervated animals caused them to initiate and complete adult development but failed to induce eclosion. Inspection of these flaccid moths revealed no new muscle formation but complete morphogenesis of all other internal organs and integumentary structures. These results indicate that muscle differentiation requires some influence derived from the central nervous system in addition to the hormonal influence supplied by the brain.

The radiation-induced inhibition of eclosion observed in *M. sexta* can be attributed to damage of abdominal ganglia and associated musculature, but this seems unlikely when one considers the aforementioned relative radioresistance of this region. Further, approximately 50% of the irradiated animals which failed to complete the emergence sequence were able to shed the abdominal portion of the pupal exuvia. The specific contribution of radiation damage to the thoracic ganglia is currently under investigation. However, the decrease in eclosion hormone activity observed in brains and corpora cardiaca of ED_{100}-irradiated animals, along with the radiosensitivity exhibited by the brain in both shielding experiments and brain

transplantation studies, suggest that radiation damage to the hormonal regulation of eclosion is involved.

In conclusion, the pattern of radiosensitivity observed in *M. sexta* during pupal-adult development reflects specific interference in the regulation of eclosion. The observed radiosensitivity is, in part, dependent upon the stage in development and decreases markedly just prior to eclosion. It is proposed that the radiosensitivity observed during early pupal-adult development (days 1–7) is in part dependent upon the endogenous ecdysone titer and may be related to a "switching on" of eclosion hormone synthesis within the brain by rising ecdysone levels. Studies utilizing brain exchange or shielding clearly demonstrate that, although the brain is a radiosensitive region, a second more sensitive component located in the thorax is also present. Lastly, although hormonally controlled, successful eclosion of nonirradiated *M. sexta* appears to require the presence of an intact brain.

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Summary

Control of eclosion in *Manduca sexta* (laboratory reared at 23–25° C on 18L:6D) was investigated by utilizing the ED_{50} (X-ray exposure required to prevent eclosion in 50% of the irradiated animals) throughout pupal-adult development as a measure of radiation sensitivity. An initial period (day 0–6) of nearly constant radiosensitivity (ED_{50} range: 13.0–14.4 kr) was followed by a brief period of increased radiosensitivity between day 6.5–7.75 (ED_{50}: 8.23 kr). Thereafter, a pronounced decrease in radiosensitivity was noted through the day of eclosion (day 8: ED_{50} = 19 kr; day 22: ED_{50} = 75 kr).

The association between hemolymph ecdysone levels and maximum radiosensitivity observed on day 7 was studied. Animals administered β-ecdysone on days 1, 4, and 12, and irradiated at various times post-injection, exhibited significant increases in radiosensitivity. Thus, radiosensitivity exhibited by *Manduca sexta* on days 0–7 is in part dependent upon the titer of ecdysone in hemolymph.

The role of the brain as a radiosensitive region was investigated in day 10 animals by selectively transplanting ED_{100}-irradiated and nonirradiated brains into ED_{100}-irradiated and nonirradiated animals. The presence of a radiosensitive component in addition to the brain is proposed since the radiation-induced inhibition of eclosion could not be completely explained in terms of brain damage alone.

Selective shielding of day 10 animals X-irradiated at an ED_{100} level demonstrated the absence of radiosensitive regions in the abdomen and their presence in both the head and thorax.

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